COGNITIVE IMPAIRMENT IN MICE EXPOSED TO ENVIRONMENTAL ARSENIC AND ITS RELATION TO PUBLIC HEALTH

by

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B. Science, Taipei Medical University, Taiwan, 2010

Submitted to the Graduate Faculty of

Environmental and Occupational Health

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Public Health

University of Pittsburgh

2013

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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Arsenic toxicity is one of World Health Organization's ten chemicals of major public health concern. Arsenic and arsenic compounds have been classified as a group one human carcinogen by the International Agency for Research on Cancer (IARC), who stated that arsenic in drinking-water is carcinogenic to humans. It is estimated that around 150 million people in seventy countries are exposed to naturally existing arsenic in polluted drinking water, and approximately sixty million people are under chronically constant exposure in Asia. Children, particularly in *in utero* or during perinatal phases, may be much more susceptible and have a higher predisposition to health effects from arsenic exposure than adults. The aim of this study is to find out how environmental level exposure to arsenic during the *in utero* phase brings alterations in epigenomic landscapes, and includes observation of differences in intellectual performance in adult mice exposed to arsenic. Since histone acetylation is dynamical and reversible, the experimental results from mice might set up references for applying to human communities that bring more knowledge for children and general public who have already been exposed to constant arsenic exposure.

The study group exposed C57BL6/J mice to 100 μg/L arsenic-contaminated water, starting one week before onset of breeding and though out the entire gestational period.. Then, chromatin immunoprecipitation combined with massively parallel DNA sequencing (ChIP-seq) was done to identify H3K9 acetylation patterns in the offspring of both the exposed and the control groups. In embryos exposed to arsenic, the arsenic caused global hypo-acetylation at H3K9 and altered functional annotation in brain tissue of the exposed mice's offspring. The study group also discovered that adult mice exposed to arsenic experienced impaired spatial and episodic memory, as well as deteriorated fear conditioning performance. The study results are the first to demonstrate how prenatal arsenic exposure brings genome wide changes in H3K9 acetylation pattern in a mice offspring; and discover an association between moderate arsenic exposure and cognitive impairment in adult mice.

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1.0 INTRODUCTION

Arsenic is pervasive in the global environment [1] [2]. It has been classified as a group one human carcinogen by the International Agency for Research on Cancer. Arsenic in drinking water is one of the main sources of human exposure and has long been a public health issue. Some arsenic compounds are released from natural objects, such as stone or minerals dissolved in groundwater [1] [2]. Moreover, usage of insecticide, excavation and mining are major anthropogenic sources of arsenic released in the US Environment [1]. In 2006, the US Environment Protection Agency (EPA) changed the maximum acceptable standard for arsenic in drinking water to 10 μg/L [3]. In the rural areas of the US, 99% of the population drinks groundwater, and they most likely still use well water that contains more arsenic than this standard allows [4]. Only 25% of bottled water in the United States is purified water, and most likely still contains heavy metals such as arsenic in concentrations elevated above the acceptable standard levels [5].

Several *in vivo* studies show that methylated forms of arsenic are likely to play a role as co-carcinogens or tumor promoters [6] [7], which point out that exposure to arsenic is linked with a higher risk of developing neoplasms of the skin, lung, bladder, liver, and kidneys [8]. Most importantly, the consequences of neurological, intellectual and behavioral health effects on human caused by chronic arsenic exposure is enormous and has long been a major public health

concern. Based on the results from several studies, which indicate that exposure to arsenic facilitates neuronal necrosis and apoptosis, [9-13] protein tau hyperphosphorylation, [14] and increased expression of the gene encoding the amyloid precursor protein [15]. The latter two proteins are involved in the formation of brain amyloid plaques and neurofibrillary tangles [16] [17]. Moderate exposures to arsenic in perinatal mice can significantly reduce corticosterone receptor levels in the hippocampus and deteriorate learning and memory performance [18]. Thus, arsenic exposure is most likely one of the causative components in the development of dementia.

There has been an increasing focus on epigenetic regulation of phenotypes to recognize and determine chronic enhancement of disease risk resulting from arsenic exposure during the embryologic period. Arsenic is an environmental toxin that brings epigenetic modifications by means of three mechanisms – DNA methylation, histone modifications, and RNA interference associated silencing [19]. A report on global changes in histone modifications noted a decreased acetylation of H3K9 observed in peripheral mononuclear cells of workers exposed to arsenic [20]. The link between changes in epigenetic signals from arsenic exposure and changes in phenotypes linked to disease later in life are not well defined. In this report, the study group presents the results of a research study undertaken to discover arsenic-induced changes in the enrichment of epigenetic marks in brain samples of offspring with *in utero* arsenic exposures. Chromatin immunoprecipitation combined with massively parallel DNA sequencing (ChIP-seq) using an antibody against acetylated lysine 9 of histone 3 (H3K9Ac) was applied to evaluate the differences in H3K9 acetylation patterns genome-wide, and to compare gene ontology terms and functional annotations between exposed and control groups. We also present the results of

behavioral testing conducted with young adult C57BL/6J mice exposed to human-relevant levels of arsenic.

2.0 REVIEW

2.1 ARSENIC

2.1.1 Source of Exposure

Arsenic is a natural constituent of the earth's crust and is pervasive throughout the environment in the soil, air, and water and most often as arsenic sulfide or as metal arsenates and arsenides. Some arsenic compounds are released into the atmosphere as the trioxide generally, and mostly by high-temperature procedures. In the atmosphere, it is primarily adsorbed by particles which are spread by winds or formed as sediment on land and water [21].

Arsenic can be released into the atmosphere and water by several means, including natural processes such as volcanic activity, dissolution of stone and minerals into groundwater, emission from botany and wind-blown dusts. Human activities, such as usage of agricultural insecticide, excavation, mining, and metal smelting, combustion of fossil fuels are major anthropogenic sources of arsenic released in the environment and are the sources of exposure, as well.

2.1.1.1 Drinking water and food

Arsenic is highly toxic in its inorganic form. The general public is exposed to increased levels of inorganic arsenic through consuming arsenic-rich water and food, applying contaminated water in food preparation and irrigation of agricultural products, industrial processes, and smoking tobacco. The most serious threat to public health from arsenic initiates from polluted groundwater. Groundwater with inorganic arsenic exists at high levels naturally in a number of countries; Bangladesh, India, China, Argentina, Chile, Mexico, and the United States, and especially Bangladesh where approximately half of the total inhabitants are at risk of using arsenic-poisoned water from tube wells. There is one estimation indicating that consumption of arsenic polluted drinking-water in Bangladesh caused about 9,000 deaths and 125,000 disability-adjusted life years (DALYs*) in 2001 [22]. *The DALY integrates the burden due to death and disability in a single index. DALY, utilizing the comparison of the burden due to diverse kinds of environmental risk factors. One DALY can be thought of as one lost year of healthy life.

Arsenic compounds are common in seafood, but are mainly found in its less toxic organic form, are not very harmful to health and are rapidly eradicated by the body [23]. Fish, shellfish, meat, domestic fowl, dairy products and cereals are also be dietary sources of exposure, although exposure level from these dietary sources are normally much less serious compared to exposure through contaminated groundwater. [23]

2.1.1.2 Industrial processes

Arsenic is not only employed industrially as an alloying element, but also in the production of glass, paint, fabric, paper, metal adhesives, timber treatment with preservatives (which might lead to soil pollution), and armament. Besides, arsenic plays a role in the hide tanning process and in pesticides, feed additives, and pharmaceutics to a restricted level. Acute arsenic poisoning seldom takes place in the workplace nowadays, exposure through industrial sources generally results in chronic health effects.

2.1.2 Magnitude of the Arsenic Exposure

Base on the information from World Health Organization (WHO), there are a number of regions where arsenic contamination of drinking-water is significant. Since the 1990s, Bangladesh has been drawing a lot of attention because of its wide occurrence of arsenic-rich water in tube wells. After an intervention operated in Bangladesh, significant progress has since been seen and the total of population who are exposed to arsenic exceeding the drinking-water quality standard in Bangladesh has decreased by an estimated 40%. In spite of these efforts, it is thought that 45 million people in Bangladesh are still at risk of arsenic exposure levels that are greater than USEPA and the WHO guideline standard of 10 μg/liter respectively [24].

The syndrome and conditions induced by long-term elevated exposure to inorganic arsenic vary from individuals, population groups, and geographical areas. Thus, there is no definite universal definition of the disease caused by arsenic. This perplexes the appraisal of the burden on health

of arsenic. Likewise, there is no method to determine cases of cancer caused by arsenic itself or from other factors. As a result, there is no dependable measure of the magnitude of this issue worldwide.

2.1.3 Acute Health Effects to Human

Acute arsenic poisoning seldom takes place in the workplace nowadays. It usually happens from accidental consumption, suicide or homicide. The lethal dose of human arsenic consumption is difficult to ascertain from case reports because it depends upon various factors, such as solubility and valence state. The immediate symptoms of acute arsenic poisoning include severe abdominal pain, nausea and vomiting, and bloody or rice-water diarrhea. These are followed by insensitivity and pricking of the extremities, muscle contraction, and death, in extreme situations. [25]

Acute neurologic effects include light-headedness, headache, lethargy, delirium, encephalopathy, convulsions, comatoseness, and sensorimotor peripheral neuropathy. It also brings harm to cardiovascular and respiratory system, such as hypotension, ventricular arrhythmia, congestive heart failure and, pulmonary edema. Hematological, hepatic, and nephritic effects are anaemia, leucopenia, thrombocytopenia, and disseminated intravascular clotting; elevated liver enzymes, hematuria, oliguria, proteinuria and acute tubular necrosis, and renal cortical necrosis. There are also other signs, such as garlic odor of the breath, and delayed appearance of Mees lines. [25]

2.1.4 Chronic Health Effects to Human

2.1.4.1 General Population

Arsenic and arsenic compounds have been classified as a group one human carcinogen by the International Agency for Research on Cancer (IARC), and have also stated that arsenic in drinking-water is carcinogenic to humans. It is estimated that around 150 million population in seventy countries are exposed to naturally existing arsenic in polluted drinking water [26].

Long-term exposure to inorganic arsenic through drinking-water and food leads to chronic arsenic poisoning. In Asia, approximately sixty million people are under chronically constant exposure.

Chronic arsenic exposure to humans mainly occurs from the ingestion of contaminated water and food. Pigmentation changes, skin lesions, and hard patches on the palms and soles of the feet (hyperkeratosis) are the first symptoms and the most distinctive characteristics. These happen after a minimum exposure of approximately five years and may be a harbinger to skin cancer [27]. Besides skin cancer, long-term exposure to arsenic may also induce lung and bladder cancers [27]. Other adverse health effects that may be associated with long-term ingestion of inorganic arsenic include developmental effects, neurotoxicity, and diabetes [27].

The Arsenic Exposure Hypothesis for Alzheimer's Disease (AD), is a hypothesis proposed by a research group after a comprehensive review of current literature combine with observations of populations at risk for arsenic exposure that suggest arsenic could be a risk factor for the development of AD (Figure 1.) [28]. If the role of arsenic exposure in AD pathogenesis is

confirmed, then AD incidence might be substantially decreased by water purification and environmental intercession [28].

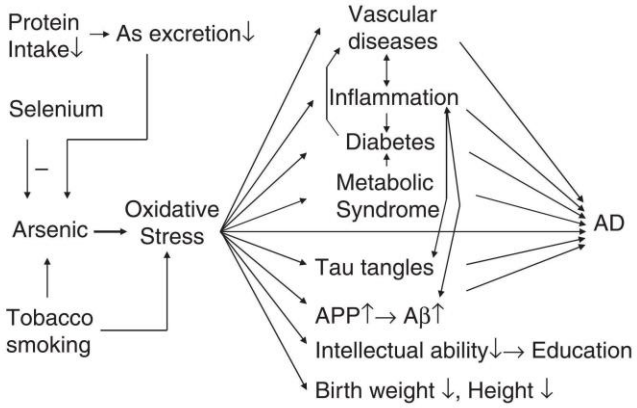

Figure 1. Hypothetic flow chart of arsenic exposure causes Alzheimer's disease (AD) [28]

The minus sign for selenium suggests that selenium reduces arsenic toxicity by enhancing arsenic excretion. Two-way arrows mean different views, that is, vascular lesion, formation of protein tau tangles, and an unusually high level of amyloid precursor protein (APP) and amyloid beta (Aß) might induce inflammatory reactions **[28].**

Tsai et al. (2003) conducted a cross-sectional study among adolescence in Taiwan indicating that long-term exposure to arsenic is likely to cause neurobehavioral effects in adolescence [29]. Another results from Gong G et al. (2010) showed that in a sample of rural-dwelling adults and elders from 434 participants that currently and long term have groundwater arsenic exposure was significantly correlated to have poorer scores in language, visuospatial skills, and executive functioning. Moreover, long-term and low-level exposure to arsenic was significantly correlated to poorer scores in global cognition, processing speed and immediate memory. The finding of a correlation between arsenic and the domains of executive functioning and memory is of crucial importance as these are cognitive domains that represent the earliest materializations of Alzheimer's disease [28].

Diabetes mellitus has been associated with an elevation of arsenic exposure in drinking water [30]. Increased prevalence of peripheral vascular disease has also been reported among residents with long-term arsenic exposure from drinking water in Taiwan [31]. Chiou et al. (1997) conducted an investigation that included 8,102 participants from 3,901 households in Taiwan. The results indicated a significant correlation between prevalence of cerebrovascular disease and human exposure to arsenic from drinking water [32]. In Taiwan, arsenic exposure has been linked to "blackfoot disease," which is a severe disease of blood vessels leading to gangrene [33]. Chen et al. (1995) examined 898 participants residing in the blackfoot-disease-endemic regions of Taiwan and suggest a possible association between long-term exposure to arsenic and prevalence of hypertension. They suggested that long-term arsenic exposure might induce hypertension in humans [34].

2.1.4.2 Children

Children may be particularly susceptible to neurotoxic substances. Children worldwide have been exposed to arsenic in drinking water at concentrations that are elevated beyond the permissible level recommended by the World Health Organization and the U.S. Environmental Protection Agency [35-37]. Children who live in regions of South Asia with high levels of arsenic such as Bengal are at particularly high risk for exposure [38] [39].

There is a study that concludes that exposure to all metabolites of inorganic arsenic can take place in the human prenatal period, meaning that different types of arsenic can be transported to the fetus during pregnancy [40]. A study from Anderson et al. (2000) also pointed out that the presence of arsenic in cord blood is hazardous because the fetus is highly susceptible to the

effects of carcinogens during the gestation phase [41]. A large retrospective study took place in two agricultural areas of Taiwan, which had low and high (up to 3.59 mg/L) water arsenic concentrations. There were 18,259 first-born singleton live births recorded and there was a statistically significant difference in birth weight of twenty-nine grams on average between exposed and non-exposed areas ($p = 0.002$) [42].

Other research results indicate that prenatal arsenic exposure is likely sex-specific, and is correlated with global DNA methylation in cord blood DNA. There was accordant positive correlation between arsenic exposure and DNA methylation among male newborns. However, arsenic exposure was by and large negatively correlated with DNA methylation among female newborns. Arsenic-caused epigenetic alterations *in utero* may potentially determine disease outcomes later in life [43].

A study group conducted a cross-sectional study among 351 participants aged five to fifteen years who were selected in West Bengal, India, from 2001 to 2003. The study result suggests that current arsenic concentrations in urine reflects all sources of recent exposure, including water and food, and were associated with small decreases in scoring in intellectual testing among school-aged children in West Bengal [44]. The other cross-sectional study from Calderón et al. (2000) was carried out on students aged six to nine who attend elementary schools in the city of San Luis Potosi, Mexico. Data on full, verbal, and performance intelligence quotients (IQ) scores, long-term memory, linguistic abstraction, attention span, and visual spatial organization were obtained through the Wechsler Intelligence Scale for Children, Revised Version (WISC-RM). The arsenic concentrations in urine were inversely correlated with verbal IQ, concepts factor

(language), and knowledge factor (verbal comprehension and long-term memory). Children having higher levels of arsenic concentrations in urine demonstrated significantly lacking performance on long-term memory and linguistic conception [45]. Children's intellectual performance can be decreased by an increase of arsenic dose. This correlation was corresponding to dose, which means that children who had more than 50 μg/L exposure had lower performed scores than children with less than 5.5 μg/L exposure [46].

2.1.5 Mouse Models of Arsenic Exposure

Waalkes et al. (2006) suggested adult mice that had been briefly exposed to arsenic during their embryologic life during a gestation period eight to eighteen days, developed tumors of the liver, ovary, adrenal gland, and lung, as well as preneoplastic lesions of the uterus and oviduct [47]. The same group reported that when expectant mice were under As^{3+} exposure in their drinking water, As^{3+} acted as a carcinogen in male offspring, which consequently developed lung, kidney, liver, and adrenal gland tumors. Moreover, when As^{3+} exposure collaborated with either *in utero* or postnatal estrogen, liver lesions were more serious and bladder lesions developed as well [48].

Ebany et al. (2008) investigated the impact of perinatal exposure to fifty parts per billion of arsenic on neuroendocrine markers associated with depression and depressive-like behaviors in affected adult C57BL/6J mice offspring. Results indicate that perinatal arsenic exposure may interrupt the regulatory interactions between the hypothalamic-pituitary-adrenal axis and the

serotonergic system in the dorsal hippocampal formation suggests that it predisposes caused offspring depressive-like behavior. These results suggest that relatively low levels of arsenic exposure during the embryologic period can have long-lasting adverse health effects on behavior and neurobiological markers correlated with these behavioral changes [49]. Corticosterone receptors are found throughout the central nervous system, particularly in the hippocampus. Martinez-Finley et al. (2009) examined a perinatal exposure to fifty parts per billion (ppb) of sodium arsenate on the C57BL/6J mice, to see the impact on corticosterone receptors and cognitive performance. Measurements of corticosterone receptors indicated that arsenic-exposed offspring reach significantly lower levels of these receptors in the nucleus than control group. Exposed offspring showed longer latency to approach a novel object than controls in an object recognition task. In the eight way radial arm maze, arsenic offspring had a significant increase in the number of entry errors compared to controls. Results suggest that moderate exposures to perinatal arsenic can significantly reduce CR levels in the hippocampus and some have significantly adverse effects on learning and memory behavior.

2.2 EPIGENETICS

Definition: ‘‘An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence.’’ This is a classical definition of epigenetics as proposed by Conrad Waddington in the 1950s, engaging the inheritance of a phenotype, capable of being passed from either mitosis or meiosis. Knowledge of the functioning result in the initiation, maintenance, and heritability of epigenetic states is an essential prospect of research in current biology. The following contents describe a set of functional actions in which such pathways can be placed, with the purpose to explain and clarify the different mechanistic prospects of epigenetic transmission.

Berger et el. (2009) proposed that there are three kinds of signals that end up in the formation of a stably inheritable epigenetic state: a signal that was proposed as the term ‘‘Epigenator,’’ which arises from the environment and activates an intracellular action; an ‘‘Epigenetic Initiator’’ signal, which reacts to the Epigenator and is essential to define the definite location of the epigenetic chromatin environment; An ‘‘Epigenetic Maintainer’’ signal, which maintains the chromatin environment in the first and successive generations. These kinds of signals are described in Figure 2 and are explained below [50].

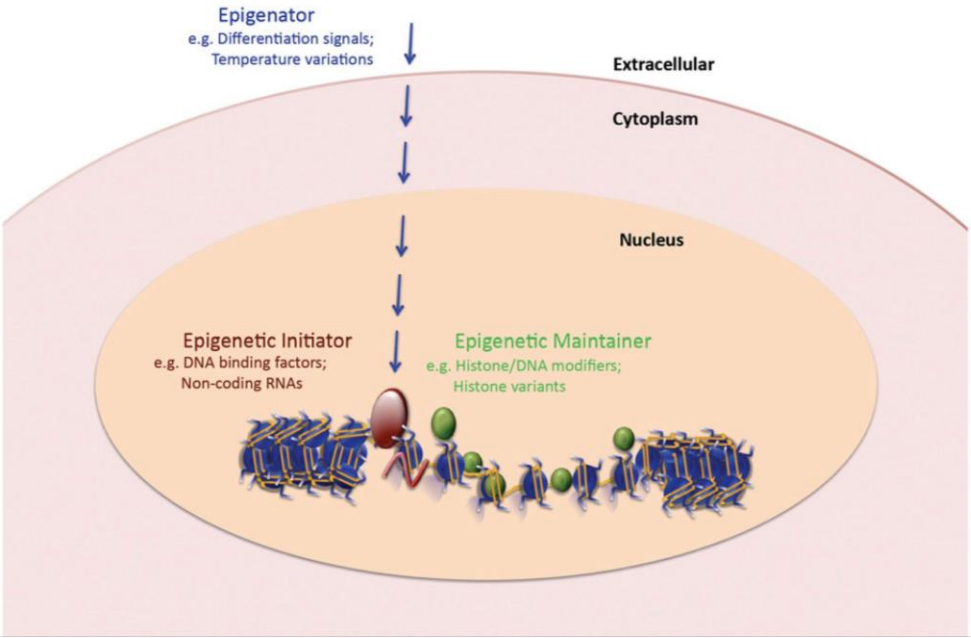

Figure 2. The epigenetic pathway.

Three kinds of signals are proposed to operate in formation of a stably inheritable epigenetic state. An extracellular signal called an ‘‘Epigenator’’ (shown in blue) introduced from the environment and can activate the starting of the epigenetic pathway. The ‘‘Epigenetic Initiator’’ (shown in red) acquires the signal from the ‘‘Epigenator’’ and is eligible to determining the definite chromatin location and DNA environment for the formation of the epigenetic pathway. The ‘‘Epigenetic Maintainer’’ (shown in green) functions to maintain the chromatin environment in the first and successive generations. Perseverance of the chromatin environment needs cooperation between the Initiator and the Maintainer. Examples for each kinds of signal are shown below the headings. Chromatin is depicted in blue [50]

2.2.1 Epigenator

The epigenetic phenotype is likely activated by changes in the environment of the cell. Every scenario takes place upstream of the first episode on the chromosome would be part of the Epigenator signal, including an environmental stimulus and the following signal pathways

directing to the Initiator. When an Epigenator signal is acquired, it is converted to an intracellular Epigenator pathway culminating in the ‘‘activation’’ of the Initiator. The Epigenator signal pathway could be a protein–protein process or a modification-based action that releases the latent activity of the Initiator. The Epigenator signal will be fleeting but staying in the cell long enough to activate the epigenetic phenotype but not necessity for successive events [50].

2.2.2 Epigenetic Initiator

The Initiator translates the Epigenator signal to mediate the formation of a local chromatin context at a definite location. Following the readying of the Initiator by the Epigenator signal, the Initiator will determine the location on a chromosome where the epigenetic chromatin state is to be formed. The Initiator could be a DNA-binding protein, a noncoding RNA, or any other object that can determine the coordinates of the chromatin structure to be congregated. Also, the Initiator will generally be a signal that acquired self-reinforcement and self-renewal through positive response mechanisms. One functioning feature of the Initiator is that it has to be sufficient to activate an epigenetic phenotype when infused into a cell. Moreover, different from the Epigenator, the Initiator may not disperse afterwards, but rather may remain with the Maintainer [50].

2.2.3 Epigenetic Maintainer

The Maintainer supports the epigenetic chromatin state but is not sufficient to activate it. This signal participates various pathways, such as DNA methylation, histone modifications,

nucleosome positioning, etc. The epigenetic maintainer signals have the mutual property but they do not possess absolute DNA sequence particularity. Therefore, the maintainers could function at any chromosomal location to which they are activated by an Initiator. Maintainers may work by transporting an epigenetic signal through the cell cycle or could maintain epigenetic landscapes in terminally differentiated cell types [50].

There are several classes of potential Maintainer signals for post-translational modifications of histone proteins, such as H3K4 and H3K27 methylation, by trithorax and polycomb complexes is related to homeotic gene expression severally. The other example, H3K9 and H4K20 methylation, is about forming memory of gene silencing. Many histone modifications function in more dynamical procedures, such as transcriptional induction and DNA repair. Therefore, histone modifications most likely function as Maintainers of epigenetic signals.

2.3 HISTONE MODIFICATIONS

Recent mechanistic studies have directly or indirectly indicated that the potential involution of modified epigenetic regulation in gene expression or cellular phenotype changes can be induced by arsenic exposure.

Histone acetylation is a dynamical and reversible action [51], lysine residues on histone tails play an essential role in the regulation of chromatin structure, gene and non-coding RNA transcription and nuclear architecture [52] [53]. Histone acetylation is modulated by two incompatible enzyme

classes, histone acetyltransferases (HATs) [54] and histone deacetylases (HDACs) [55]. Histone acetylation on lysine residues leads to the starting of a transcriptionally competent environment by reducing the affinity of DNA to the acetylated amino termini of histones, allowing access of general transcription factors [56] [57]. Moreover, acetylation of histone H3 is also correlated with functional enhancers [58] [59].

One study shows that chronic Arsenic exposure in human adults from Araihazar, Bangladesh increases H3K9me2 and H3K27me3 in females, which represents transcriptional repression, and a decrease in H3K9 acetylation in males only [60]. Additionally, several previous studies have shown that arsenic exposure induces the reduction of acetylation in histones H3 and H4, increases acetylation in H3K9ac, H3K4me2, and H3K4me3, and causes loss of H3K27me3, as well as loss or gain H3K9me2 [61-71]. Another study indicates that human peripheral blood mononuclear cells (PBMCs) or white blood cells can be employed to evaluate the correlation between metal exposure and post-translational histone modifications (PTHMs). Furthermore, plenty of chromatin silencing marks may service for the silencing of tumor suppressor genes which can predispose to cancer developing progress. This indicates that H3K9 hypo-acetylation might play a vital role in establishing DNA methylation [72].

Also, studies have shown that AsIII exposure induces elevated histone acetylation, which was reportedly responsible for the up regulation of genes involved in apoptosis or the response to cell stress after exposure to arsenic [73] [74]. AsIII has been shown to inhibit HDAC genes that correlate with increased global histone acetylation [75].

Other research indicates that $NaAsO_2$ exposure increases global histone acetylation significantly. This effect was associated to the inhibitory of the histone deacetylase (HDAC) function because $NaAsO_2$ was in a position to suppress HDACs comparable to the HDAC inhibitor trichostatin A (TSA). Moreover, the research result suggests that $NaAsO_2$ leads to chromatin opening by histone hyperacetylation due to HDAC inhibitory and the increase of the ability of nucleosome-associated proteins. As the chromatin compaction is crucial for the regulation of gene expression as well as for genome stability, therefore, chromatin opening by $NaAsO_2$ may play a significant role to impart its genotoxic effects [76].

The combination of these observed histone modifications by arsenic exposure, is highly correlated with global transcriptional modification and may be utilized as a biomarker of arsenic exposure.

3.0 ANALYTICAL SECTION

Chronic arsenic toxicity in drinking water has long been a serious public health threat worldwide, and exposure may cause intellectual dysfunctions, and neurological and cognitive deterioration. Children may be much more susceptible to this neurotoxic substance, particularly if it is *in utero* or during the perinatal phases. One study concludes prenatal exposure to arsenic in water brings alterations in epigenomic landscapes. Several studies have shown that cognitive and learning deficits exist in children with embryologic exposure from environmentally relevant concentrations of arsenic.

The following data presented in this section provides the fact that prenatal arsenic exposure changes in epigenic landscape in mice offspring. These experimental results can play an important role as relevant model systems on how the general public's health is affected by arsenic, especially when addressing prenatal and children's health and intellectual performances.

The results presented in the analytical section were performed in Dr. Koldamova and Lefterov laboratory and were recently published by Cronican et al. [84]. I have participated in tissue collection and performed some of the techniques documented in my Master project.

3.1 PRENATAL ARSENIC EXPOSURE EFFECTS BIRTH WEIGHT.

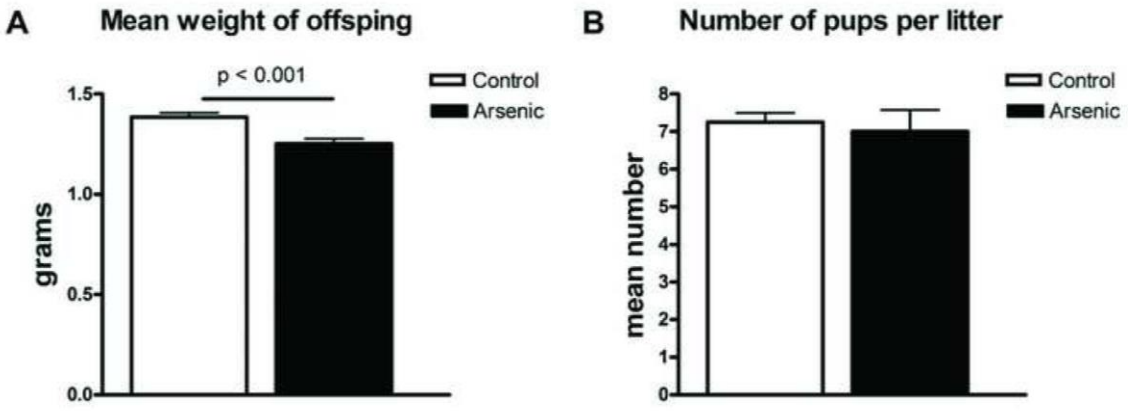

Figure 3. Pups' weight decreased by prenatal exposure to arsenic [84]

Figure 3: **A.** The mean weight of the pups is decreased approximately by 10 % in arsenic treated mothers. $P < 0.05$ by t-test. **B.** Number of pups per pregnant dam is not significantly changed by arsenic treatment.

Figure 3. "Pups' weight decreased by prenatal exposure to arsenic" is from Cronican et al. Base on the information from figure 3, female mice were initially provided with either 100μg/L arsenic in spring water (arsenic exposed) or plain spring water (control) for one week. Males were then added to the cage and removed one day after the first introduction. Females continued under exposure for the duration of their pregnancy. Pups were collected within 24hrs of birth and litter size and weight recorded. Although differences in litter size were not observed and there were no visible gross anatomical changes of P1 pups, there was a significant difference between the average individual birth weight of arsenic exposed (1.25±0.02 grams) and control (1.38±0.02 grams) offspring.

3.2 GENOME-WIDE MAPPING OF H3K9 ACETYLATION IN OFFSPRING

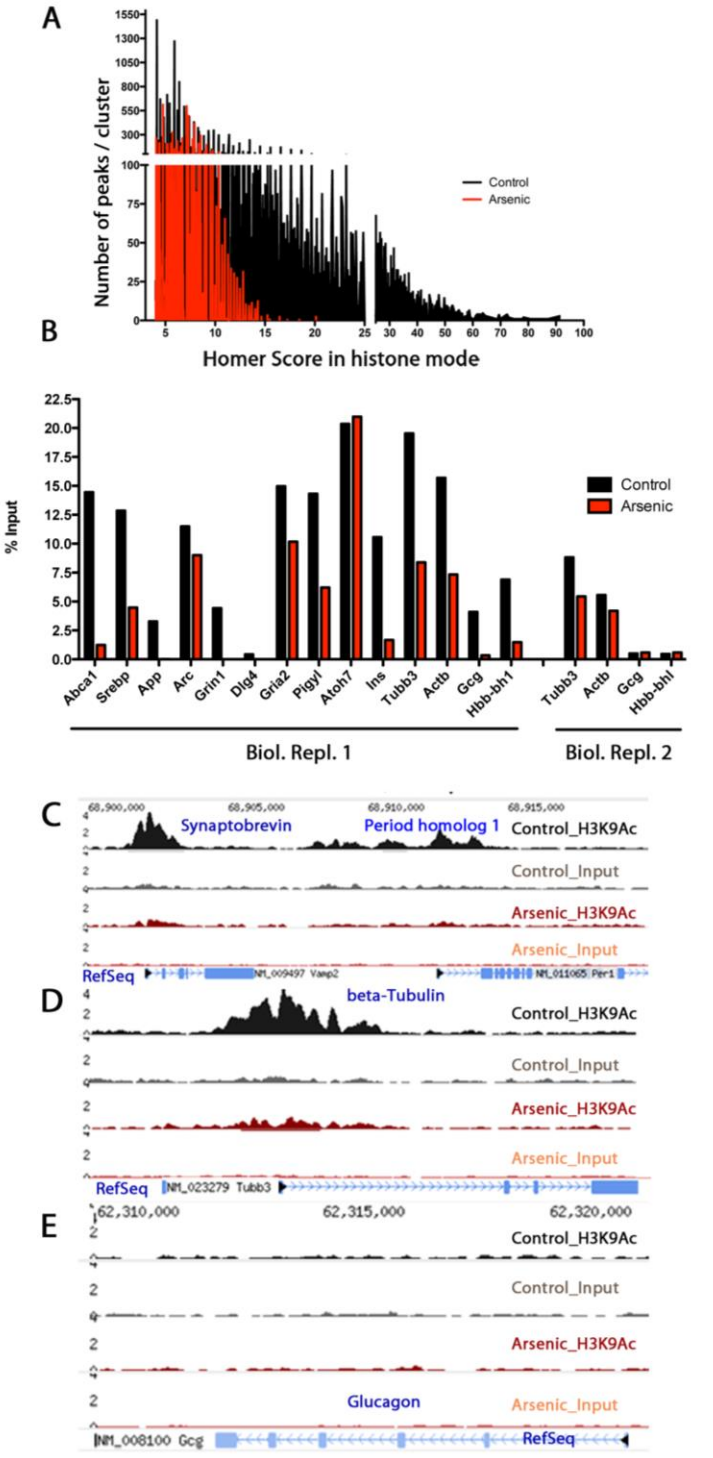

Figure 4. Global hypoacetylation at H3K9 in response to arsenic exposure during embryonic life [84]

Figure 4. “Global hypoacetylation at H3K9 in response to arsenic exposure during embryonic life” is from Cronican et al, and it indicates that each of the clusters on the histogram in Panel **A** represent all significant peaks of enrichment with the same HOMER score from arsenic (in red 6.71 median, 6.86 average, 4.03 min, 20.13 max, 8577 total peaks) and control (in black: 9.07 median, 11.70 average, 4.02 min, 91.62 max, 37946 total peaks) treated samples.

B. To further confirm the differences in global acetylation we calculated and compared the enrichment of H3K9Ac as % of input using primers in the proximal promoters of several genes. ChIP-QPCR validation assays from two biological replicates demonstrate differences in H3K9Ac enrichment in the proximal promoters of randomly selected genes from arsenic and control treated samples. Abbreviations: Abca1, ATP- binding cassette, sub-family A, member 1; Srebp, Sterol regulatory element binding transcription factor 1; App, Amyloid precursor protein; Arc, activity regulated cytoskeletal-associated protein; Grin1, Glutamate receptor, ionotropic, N-methyl D-aspartate 1; Dlg4, Discs, large homolog 4; Gria2, Glutamate receptor, ionotropic; Plgyl, Phosphatidylinositol glycan anchor biosynthesis, class Y-like; Atoh7, Atonal homolog 7; Ins, Insulin; Tubb3, β-Tubulin; Actb, β-Actin; Gcg, Glucagon; Hbb-bh1, Hemoglobin Z, beta-like embryonic chain.

C, D, E. Screen shots taken from gene browser TessLA (http://ngsc.med.upenn.edu), demonstrate the difference between the levels of H3K9Ac enrichment in the proximal promoters of several genes. Genes, used throughout the study as positive - Tubb3 (expected enrichment), and negative - Gcg (no enrichment) controls for quality validation of the ChIP samples, as well as some randomly selected genes (Vamp2 and Per1) are shown.

3.2.1 Overview of a CHIP–sequencing Analysis and Work Flow

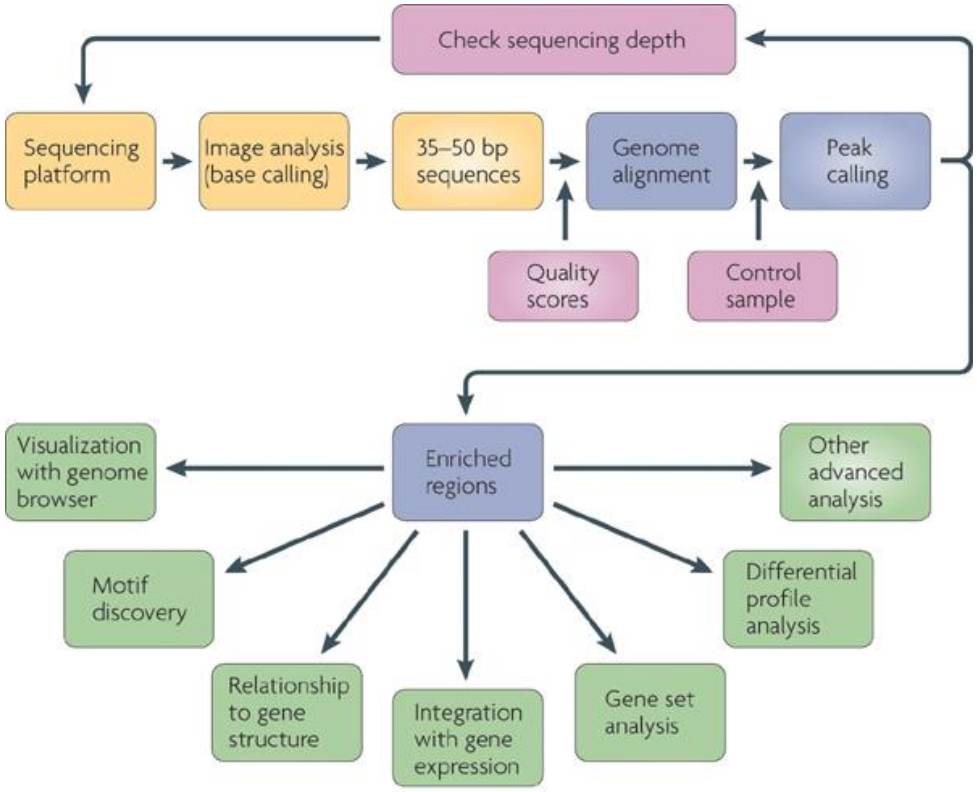

Figure 5. Overview of CHIP–sequence analysis [77].

ChIP-Sequence is a mixture of chromatin immunoprecipitation (ChIP) with ultra high-throughput massively parallel sequencing, a leading experimental method to analyze protein interactions with DNA, figure 5 shows the overview of CHIP-sequence analysis. ChIP-Seq is employed to study genome-wide landscape and allows mapping of protein–DNA interactions *in vivo* on a genome degree. It costs less, but with higher accuracy and more efficient than other technologies. Moreover, in just one experimental step, measurement of entire genome modifications in transcription-factor binding that responds to environmental stimuli. Figure 6 displays the experimental work flow of CHIP-sequence.

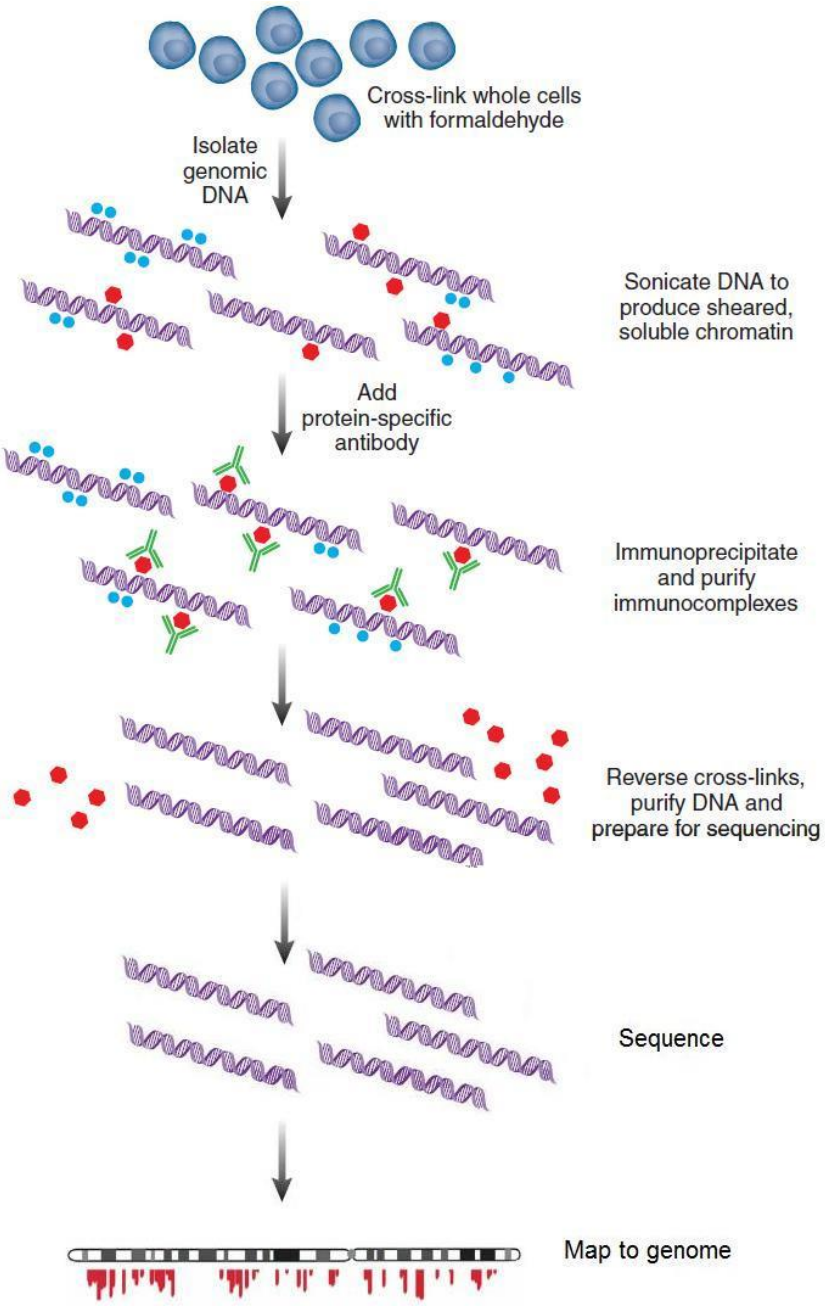

Figure 6. Work flow of CHIP-Sequence [78].

3.3 MEMORY AND COGNITIVE IMPAIRMENTS IN EXPOSED MICE

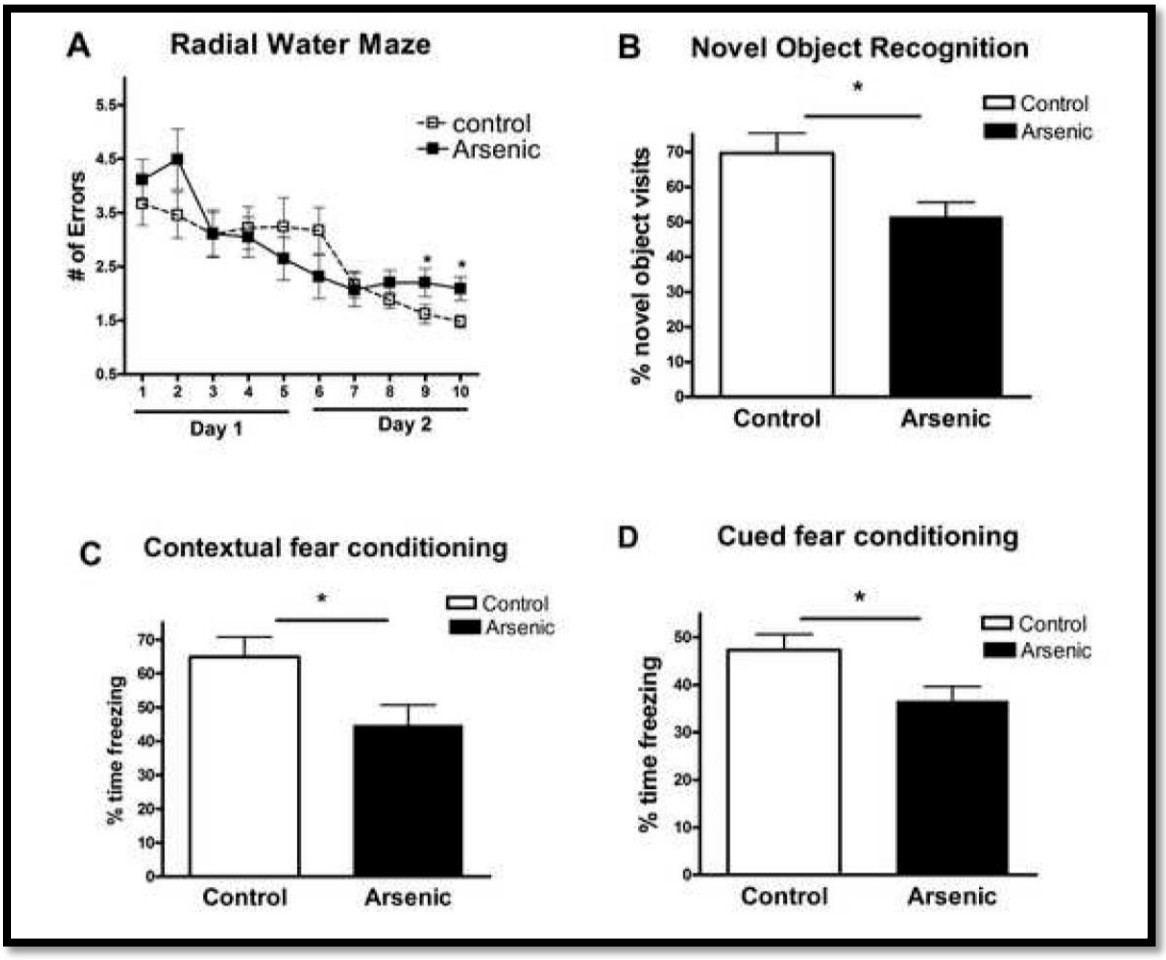

Figure 7. Arsenic treatment causes memory deficits in mice [84]

Figure 7 "Arsenic treatment causes memory deficits in mice" from Cronican et al, provides the experimental results of six month old C57BL6/J mice exposed to 100 ppb arsenic supplied in the drinking water for three weeks (N=15). Control mice received plain water (N=14). At the end of the treatment, arsenic treated and control mice were subjected to different behavior paradigms to assess cognitive performance. **A**. Radial water maze demonstrates that the spatial memory is affected by arsenic on the last two trial blocks. The radial water maze (RWM) is consists of six

swim arms stretching out from an open central area, with an escape platform located at the end of one specific arm, called the goal arm. The goal arm location stays the same for a given mouse. The mouse supposed to enter the goal arm. Otherwise, entering into an incorrect arm gets as an error count. The RWM possess spatial complexness and achievement measurement simplicity of the dry radial arm maze combined with the instant acquisition and strong motivation observed in the Morris water maze [79]. **B.** Novel object recognition shows a significant difference between arsenic and control group of mice. Mice posses a disposition to interact more with a novel object than with an accustomed object. This disposition has been adopted by behavioral scientists and neuroscientists to study learning and memory. The novel object recognition (NOR) task is a model adopted to measure non-spatial memory deficit, and for studying cognitive deficit symptoms in rats [80].

C and D. Arsenic treatment significantly impairs contextual (C) and cued fear conditioning (D). Data are presented as means ± SEM and analyzed by t-test. *, $p < 0.05$.

The obtainment of conditioned fear behavior was estimated by measuring 'freezing', a feature of defensive position when stimuli signal to occur that predict danger [81]. Here we explore the relation between arsenic exposed mice and memory in fear conditioning.

4.0 DISCUSSION

Evidence in the review section and analytical section have illustrated that arsenic acts through different epigenetic functions. The delineation of genome-wide patterns of DNA methylation, posttranslational histone modification and mRNA expression after arsenic exposure *in utero* and *in vivo* interprets a new boundary toward our understanding of the mechanisms of arsenic neurotoxicity. The study results from mice are relevant or correspond to the studies showed in the review section about human being, such as prenatal arsenic exposure reach statistically significant difference in birth weight between exposed and control in both mice offspring and human live births. The studies in the review section show that children who chronically expose to arsenic are lacking performed on long-term memory and linguistic skill; chronically exposed adults and elders have poor scores in language, visuospatial skills and immediate memory. These studies in human are relevant to the results that provided in the analytical section.

There are some limitations in the study. While experiments in relevant model systems could supplement the human studies as a review part, there may be divergences between epigenetic alterations in animals and humans, and between assorted tissues and cell phenotypes. Moreover, human populations assessed in the present studies came from a population that has a complex mixture of chronic exposure to inorganic arsenic, including inhalation, ingestion, and dermal

exposure. In contrast, the mice used in the study came from mothers who were exposed orally to arsenic and for days only. Therefore, studies in human populations exposed to arsenic in drinking water will be required to comprehend how individual differences in arsenic methylation and genetic background, as well as environmental factors such as diet, age, and levels of other water contaminants influence the epigenetic response to chronic arsenic exposure. Studies will also be necessary across assorted tissue and cell types to recognize and corroborate the levels and patterns of epigenetic markers in these cells.

The pervasiveness of arsenic in the environment raises the importance of assessing the neurobiological consequences of exposure to environmental levels of this heavy medal. Up to now, when starting an investigation to find out the association between arsenic exposure and disease outcomes, most animal studies have been focusing on either perinatal exposure, or very high levels of arsenic exposure. These unusually high levels are linked to a quantity of symptoms indicating toxicity, such as increased mortality, and they do not corresponding demonstrate precise effects of environmental levels of arsenic exposure in humans where such symptoms of toxicity are unavailable. In this study, prenatal exposure model in adult mice provides a unique opportunity to demonstrate the effects of arsenic during embryogenesis at an environmentally relevant level, magnitude lower than what has been previously studied, we can find out disease outcomes and mechanisms associated with environmental arsenic exposure in drinking water. Moreover, in our study with the 100 ppb low levels examined, we did not discover any evidence of overt toxicity or increased mortality and weight loss of exposed adult mice is unavailable. However, the mice exposed to environmentally relevant levels of arsenic for a relatively short period of time demonstrate cognitive impairment in a battery of behavioral tests.

The cost of prevention and early intervention are most likely much lower than the costs of proving treatments. Early exposure in childhood or even *in utero* phase may cost more for paying medications and bring down the productivity of the country. The most crucial intervention in exposed areas is the prevention of further exposure to arsenic by the supplying of a hygienic, harmless water source for drinking, food preparation and irrigation for agriculture.

With the replacement of high-arsenic supply with low-arsenic, microbiologically safe provisions such as collect rain water and processed surface water. Low-arsenic water can be a resolution for drinking, cooking, and irrigation purposes in arsenic-contaminated area. At the same time, high-arsenic water can be used for other aims such as handwashing, showering and cleaning laundries [82].

Distinguish between high-arsenic and low-arsenic sources can be an effective action as well. For instance, screening water for arsenic concentrations and paint tube wells or hand pumps in different colors. This can be an effectual and low-cost ways to rapidly lessen exposure to arsenic when accompanied by effectual education [82].

Install arsenic removal devices for either community-based or domestic levels, and assure the suitable elimination of arsenic. Practical applications for arsenic elimination include absorption, ion exchange, oxidation, coagulation–precipitation, and membrane techniques. Long-term interventions are also needed to lessen occupational exposure levels from industrial manufacturers. Education and community participation are also one of the fundamental ingredients for achieving successful interventions, arouse both the general public and the health establishments' awareness the harmful consequences of high arsenic intake and how to avoid it.

Targets possessing higher probabilities of exposure to arsenic-rich sources should also be monitored for early signs of arsenic poisoning – usually skin problems. It should be noted that total urinary arsenic does not differentiate between inorganic arsenic, which is toxic, and organic arsenic, some of which is not [82].

WHO's reactions to lower arsenic exposure consists of establishing standard values, follow up evidences and accommodating risk management guidance. The Guidelines for Drinking-Water Quality published by WHO are planned as the basis for regulation and standard setting internationally, a current version of guideline value for arsenic is 10 μg/liter. The guideline value is provisional because of assessment complications and the practical complexity in eliminating arsenic from water [83].

5.0 CONCLUSION

In conclusion, findings in the present study indicate a relationship between arsenic exposure during embryogenesis and genome-wide hypo-acetylation at H3K9; and a significant difference between the average individual birth weight of arsenic exposed and control offspring. The data presented in this study also clearly demonstrate the intellectual and behavioral consequences of environmental level arsenic exposure. Assuming that many adult diseases have a fetal origin, further studies in the future should be detailing the molecular and dynamical pathways of prenatal arsenic exposure on the alterations in epigenomic landscapes, and how to reverse the processes are essential to our children and public health.

Although plenty of laboratory studies have contributed valuable knowledge about adverse health effect of environmental exposure to arsenic, to give our children and general public a friendly environment to live in healthily, we hope that the present findings add a new sense of urgency to efforts aimed at alleviating, and eliminating chronic arsenic exposure, combine with the enforcement of the earliest and the most effectual intervention to the exposed area to prevent further exposure to arsenic in the world, especially in developing countries is also crucial.

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